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EXTENT OF SKELETAL INVOLVEMENT DID NOT PREDICT FOR OUTCOME AFTER AUTOLOGOUS STEM CELL TRANSPLANT FOR PATIENTS WITH MULTIPLE MYELOMA

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Background: Autologous stem cell transplant lengthens survival in patients with multiple myeloma, but has mortality from infections and high costs. Determination of prognostic factors predicting prolonged survival prior to transplant may help determine which patients may be better candidates for autologous SCT. Here, we hypothesized that extensive skeletal involvement prior to transplant predicted for adverse transplant outcome.

Methods: Patients undergoing autologous stem cell transplants from 1999 to 2003 at Rush University Medical Center were reviewed to determine if having fewer lytic lesions prior to stem cell transplant there would lead to longer disease free survival post-transplant. Patients with less than 3 lytic lesions were compared to those with more than three lytic lesions regardless of subtype of myeloma or time to transplant. 83 patients had autoSCT at RUMC during the time frame, 12 patients did not have documented skeletal surveys and one patient underwent alloSCT, so they were not included in the analysis. Of the remaining 70, 44 patients had numerous lesions while 26 had 0–2 lesions. The mean age at SCT for both groups was 55.6 years. The various subtypes of myeloma for the group of 44 patients with numerous lesion is 25 with IgG, 6 with IgA, 1 with IgD, 5 with light chain, and 7 that were not specified, and for the group of 26 patients with 0–2 lesions, 19 were IgG, 2 IgA, 4 not specified and 1 with POEMS. The average time to transplant from diagnosis was 624 days with a range of 117–2294 for the group with 0–2 lesions, and 511 days with a range of 93–7321 days for the group with numerous lesions.

Results: 28/44 patients with numerous lesions had documented disease progression with a median of 27 months from transplant, with ranges of 4–74 months, while 19/26 patients with fewer lesions progressed with median of 35 months and range of 12–75 months. Prior to August 2008, 15/44 patients in the group with numerous lesions died, while 19/26 patients in the group with fewer lesions died. Analysis with Kaplan Meyer survival curves showed no difference in disease free survival and death between the two groups, with p-values of 0.34 and 0.53 respectively. The estimated 5-year survival was 60% versus 50% for pts with 0–2 lesions versus numerous lesions respectively.

Conclusions: We conclude that extent of skeletal involvement defined by number of lytic lesions does not predict for transplant outcome in patients with multiple myeloma.

GRAFT PROCESSING

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DEVELOPMENT OF A SEGMENT-BASED ALDEHYDE DEHYDROGENASE ASSAY TO DETERMINE UMBILICAL CORD BLOOD UNIT (CBU) POTENCY

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Background: Banked, unrelated CBU provides access to transplantation for patients lacking matched donors. However, after CB transplantation (UCBT), up to 20% of patients experience primary graft failure, suggesting that rapid assays to assess CB potency are needed. Preliminary clinical studies demonstrated that post-thaw colony forming units (CFU) predict neutrophil and platelet engraftment as well as overall survival but, time (14 days) and variability limit their usefulness as a potency assay. CFUs in fresh cord blood are highly correlated with the number of cord blood cells expressing aldehyde dehydrogenase ($ALDH^{br}$), an enzyme enriched in hematopoietic progenitor and stem cells. Therefore, we developed an assay for $ALDH^{br}$ cells in segments attached to CBUs to measure potency for UCBT.

Methods: Fresh CBUs, donated to the Carolinas Cord Blood Bank at Duke, were selected based on $ALDH^{br}$ content (high~1.0%, medium~0.4%, low~0.1%) and divided into segments (~100 ul) for cryopreservation and post-thaw testing. Thawed segments were washed to remove the DMSO with 5% dextran/albumin (DA) or 1% human serum albumin/phosphate buffered saline (HSA/PBS). The segments were assayed for CFU [Methocult®], $ALDH^{br}$ [Aldefluor®], viability, expression of CD34, CD45 and Glycophorin A [flow cytometry] and total nucleated cell (TNC) count [Sysmex], and total number of each cell population in the segment pre-cryo and post-thaw was calculated.

Results: $ALDH^{br}$ cells (~85% CD34/45 positive) were reproducibly assayed by flow cytometry in fresh and frozen segments in less than a day regardless of $ALDH^{br}$ percentage. DA and HSA/PBS washes reliably removed DMSO with little loss in the number of CFU or $ALDH^{br}$ cells, although TNC count was reduced. Ammonium chloride lysis of red blood cells post-thaw reduced CFU and $ALDH^{br}$ cells, so an alternate procedure based on exclusion gating of GLY A+ cells was adopted. Studies on segments derived from the same cord showed that the number of $ALDH^{br}$ cells could be reliably measured and that pre-cryo and post-thaw changes in CFU and $ALDH^{br}$ cells strongly correlated.

Conclusions: $ALDH^{br}$ cell content of thawed CBU segments can be measured rapidly and reliably by flow cytometry. Post-thaw $ALDH^{br}$ correlates with CFUs. Since post-thaw CFU values predict engraftment, we propose further studies of a rapid, post-thaw assay of $ALDH^{br}$ content that could serve as a predictor of CBU potency for neutrophil and platelet engraftment and overall survival after UCBT.

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SIGNIFICANT EX-VIVO EXPANSION OF CORD BLOOD (CB) NATURAL KILLER (NK) CELLS AND CONCOMITANT DECREASE IN CB T-CELLS BY GENETICALLY REENGINEERED K562 CELLS (K562-MBIL15-41BBL)

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The limitations of the use of NK cells in adoptive tumor immunotherapy include lack of tumor recognition by NK cells and limited numbers of functionally active NK cells (Shereck/Cairo et al, Pediatr Blood Cancer, 2007). To circumvent these limitations, methods to expand and activate peripheral blood NK cells have been developed (Imai et al, Blood, 2005). We previously reported the successful expansion and functional activation of cord blood NK cells by ex-vivo cellular engineering with a cocktail of antibody and cytokines (Ayello/Cairo et al, BBMT, 2006). In this study we sought to develop an improved CB NK cell expansion system for use in adoptive cellular immunotherapy strategies. Freshly isolated CB mononuclear cells (CBMC) were cultured with modified K562 cells expressing membrane bound IL15 and 4-1BB ligand (K562-mbIL15-41BBL; Imai et al, Blood, 2005). After irradiation with 100Gy, K562-mbIL15-41BBL cells were incubated in a 1:1 ratio with CBMC + 10 IU/mL rhIL-2 for 7–14 days. CD3 and CD56 expression was determined by flow cytometry at Days 0, 7 and 14. On Day 0, CBMC included a population of CD56+/CD3- NK cells of 3.9% ± 1.3%. After 7 days of culture with K562-mbIL15-41BBL cells the percentage of NK cells increased to 71.7% ± 3.9%, as compared to 9.7% ± 2.4% in cultures with media alone and 42.6% ± 5.9% in cultures with wild-type K562 cells ($p<0.01$). Concomitantly, there was a significant decrease in CB T-Cells vs culture with wild-type K562 or media alone (15.1 ± 1.7% vs 35.7 ± 2.4% vs 51 ± 7.1%, respectively) ($p<0.001$). Overall, this represented a 35-fold or 3374% ± 385% increase of the input NK cell number. This was significantly increased compared to culture with wild-type K562 (1771% ± 300%, $p<0.05$). On Day 14, there remained a significant increase in NK cell populations between CBMC incubated with modified K562 cells compared to wild-type K562 cells and media alone (62.0% ± 2.1% vs 27.9% ± 2.4% vs 5.5% ± 0.4%, $p<0.001$). A standard cryopreserved UCB unit (25 mL) contains approximately 750×10^6 MNCs. By using the smaller 5mL aliquot (20%) of a two-aliquot bag (150×10^6 MNCs x 3.9% = 5.8×10^6 NK cells), this expansion method would hypothetically yield approximately 200×10^6 CB NK cells after 7 days of culture. In summary, we have